PATENT

Attorney Docket No. 015389-002600US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	)	
	)	
Thomas R. Cech et al.	)	Examiner: Not Assigned
	)	
Serial No.: 08/912,951	)	Art Unit: 1815
	)	
Filed: August 14, 1997	)	
	)	INFORMATION DISCLOSURE
For: HUMAN TELOMERASE CATALYTIC SUBUNIT: DIAGNOSTIC AND THERAPEUTIC METHODS	)	STATEMENT UNDER 37 C.F.R. § 1.97 and § 1.98

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

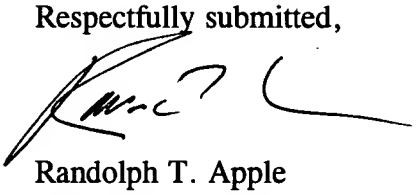
The references cited on attached form PTO Form-1449 are being called to the attention of the Examiner. Copies of the most closely related published references (references AL, AM, DR, DS, EA, and FA-FS) are enclosed and are discussed in the *Detailed Discussion of Most Closely Related References* filed herewith together with a *Petition to Make Special Under 37 C.F.R. § 1.102(d).* The remaining references listed in the form PTO Form-1449 are not being provided pursuant to 37 C.F.R. § 1.98(d). They were provided in U.S. Application Serial No. 08/724,643, filed October 1, 1996, a prior application to which the above-referenced application claims priority.

It is respectfully requested that the cited information be expressly considered during the prosecution of this application, and the references be made of record therein and appear among the "references cited" on any patent to issue therefrom.

As provided for by 37 C.F.R. § 1.97(g) and (h), no inference should be made that the information and references cited are prior art merely because they are in this statement.

Applicants believe that no fee is required for submission of this statement, since it is being submitted prior to the first Office Action on the merits.

Respectfully submitted,



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November 19, 1997

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USSN 08/912,951

Appendix I to Statement Regarding Pre-Examination Search

RE: PRE-EXAMINATION SEARCH IN SUPPORT OF PETITION TO MAKE SPECIAL:
S/N 08/854,050
HUMAN TELOMERASE
YOUR REF: 015389-002940
PAS NO: 97-13259

Dear Ted:

Pursuant your authorization and instruction of October 21, 1997 the above patent search has been conducted at the United States Patent and Trademark Office and using databases. An appendix listing several exemplary claims; a PTO-1449; an excerpt from an information disclosure statement for an ancestor application (S/N 08/724,643); three scientific publications and the human telomerase reverse transcriptase (hTRT) cDNA and protein sequences were enclosed with your letter of October 21, 1997.

noted
JLS
9/25/98

The search was directed toward showings in prior art of telomerases. More specifically the search was directed toward the inventive combination of the characterization, DNA and amino acid sequences of the human catalytic protein subunit, referred to as telomerase reverse transcriptase (TRT). The recombinant protein preparation (or nucleic acids, anti-TRT antibodies) is used in diagnosis and treatment of disease; in addition, its reconstituted activity is used in the screening of test compounds for the treatment of diseases. Further details were presented in your letter; note was made that the search was to include references up to August 14, 1997.

end page one/page two follows...

The following paragraphs detail the search and our findings. The work was organized first to locate Category I findings which show the above described invention directly in a document; second to locate Category II findings that offer predictions of human telomerase reverse transcriptase subunit characteristics; third to locate Category III findings that mention the need or desirability to characterize this subunit; and finally Category IV documents that are directed to analogous art such as telomerase homologs, reverse transcriptases in general, and other characteristics of human telomerase. **We have considered hundreds of documents located in our search activity and we have not located showings of the subject human TRT.** All of the documents presented below fall into Categories III and IV.

In summary, the search was organized into the following activities:

- Manual PTO class search of U.S. patents;
- Automated Patent System (APS) Computer full text search of U.S. patents and European and Japanese titles and abstracts;
- Foreign Patent Access System (FPAS) Computer title search of foreign materials
- The following databases were searched on STN for preparation, diagnostic and therapeutic applications of telomerase:

US Patents	1972-present
World Patents Index	1963-present
Patosep(European patents)	1978-present
Patoswo(World patents)	1983-present
Medline	1966-present
Biosis	1969-present
Scisearch	1974-present
Embase	1974-present
Chemical Abstracts	1967-present
NLDB(Newsletter database)	1988-present

- Search of NIH grant awards database
- Reference library look-ups.

The documents called out in attachments described below have been selected for inclusion in this report. As explained below, some are included as whole documents and other have only abstracts.

Attachment A U.S. Patents and Foreign Documents;
Attachment B Literature; and
Attachment C NIH Grant Abstracts.

end page two/page three follows...

Attachment A begins with a first index page which lists the documents; one copy of each is enclosed for Attachment A. In addition, Attachment A includes abstracts for the listed documents. Similarly, the first index page of Attachment B lists publications which are then attached with abstracts. Attachment C is a collection of abstracts; they are not organized on an index page.

The following comments are directed to documents located in **Attachment A.**

VILLEPONTEAU ET AL., USP 5,583,016 (GERON CORP) provides recombinant mammalian telomerase (column 3, line 31) and discusses purification of the protein (including human) components.

WO 96/40868 shows telomerase activity in a truncated vertebrate telomerase in which the protein subunits may be produced by recombinant means, synthesized or obtained from natural sources. This protein is used in diagnostic or therapeutic methods, and in assays for telomerase.

WO 96/12811 shows eukaryotic recombinant gene products which include telomerase RNA templates, proteins, polypeptides and peptides associated with telomerase.

WO 96/19580 shows the mammalian p80 subunit telomerase polypeptide homolog.

The following comments are directed to documents located in **Attachment B.**

RHYU (page 892) highlights the need to identify the protein components of human telomerase and suggests that conservation of the protein sequences from lower organisms may serve to probe for the mammalian telomerase protein.

The following comments are directed to **Attachment C.**

Several research proposals submitted to and funded by NIH, recognize the need to characterize by mapping and cloning, the genes for human telomerase proteins. These grant numbers include 5 U19 CA 67760-02 Bradford, R37 AG09383 Greider, U19CA67760 Von Hoff, and 1 F32 AG05781-01 Epstein.

The remaining references in the attachments are of general interest in the area of telomerase-associated proteins; telomerase activity assays and reverse transcriptases.

end page three/page four follows...

For your information the manual search included the following Patent Office classifications:

Class 424 (Drug: Bio-Affecting and Body Treating Compositions)
Subclass 94.5 (enzyme containing transferases)
Subclass 146.1 (immunoglobulin, antiserum, antibody binding enzyme)

Class 435 (Chemistry: Molecular Biology and Microbiology)
Subclass 7.4 (measuring/testing assay to identify enzyme)
Subclass 15 (involving transferase)
Subclass 183 (enzyme; proenzyme; composition thereof; process for preparing)
Subclass 194 (transferring phosphorus containing group)

Class 536 (Organic Compounds)
Subclass 23.2 (DNA/RNA sequence encodes an enzyme)
Subclass 25.1 (3'-5' linked RNA)
Subclass 25.2 (2'-5' linked RNA)

Class 530 (Chemistry: Natural Resins or Derivatives: Peptides or Proteins)
Subclass 387.1 (immunoglobulin, antibody, or fragment thereof)
Subclass 388.26 (human monoclonal..binds enzyme).

The above subclasses were NOT manually searched additionally for foreign art and publication references. However, the Foreign Patent Access System (FPAS), discussed below, and the Word Patent Index databases covered this area.

The search also included the APS Computer full text search (1970 to date) for various "key words" and character fields. The following search statements (or combinations thereof) were conducted under the U.S. Patent file: telomerase?; ribonucleoprotein?; reverse transcriptase?; human. These key words were defined to appear anywhere in the text of the documents. The same search statement was conducted under the European and Japanese Abstract files. The information was downloaded and reviewed by subject title and abstracts so that any relevant selections could be made.

The search additionally included the FPAS Computer title search for various "key words".

A Medline database search gave rise to some of the references listed in Attachment B. Publication look-ups were carried out at the National Institutes of Health Clinical Center library (Bethesda, MD) and Dahlgren Memorial Library, Georgetown University Medical Center.

end page four/page five follows...

The following examiner of the art unit indicated was consulted regarding the field of search:

PE ROBERT WAX (1814).

His name was given to us by Examiner Lila Feisee. In an effort to assure technical sufficiency of this search a consultation was made with Primary Examiner Wax with respect to the subclasses to be searched. He confirmed that the subject matter presently claimed belongs in many of the class/subclasses searched above.

For your information, Dr. D.J. Hansell (PhD. Oxon.) conducted part of this search.

The enclosures to your letter (including scientific publications) and diskette with PTO-Form 1449 and Information Disclosure Statement are returned.

Kindly call if you have a question.

Very truly yours,

PRIOR ART SEARCHES, INC.

Tommy L. Foster

TLF:DJH:djh:o13259r
Enclosures

ATTACHMENT A

U.S. Patents:

5,489,508 WEST ET AL
5,583,016 VILLEPONTEAU ET AL
5,597,697 DIAMOND

Foreign Documents:

JP 09154575-A Soosie K.K., Japan
WO 96/12811 Arch Development Corp., U.S.
WO 96/40868 Cold Spring Harbor Lab., U.S.
WO 96/19580 Cold Spring Harbor Lab., U.S.

United States Patent **5,597,697**
Diamond **Jan. 28, 1997**

Screening assay for inhibitors and activators of RNA and DNA-dependent nucleic acid polymerases

Inventors: Diamond; Paul (693 Somerville Ave., Apt. 4, Somerville, MA 02143).

Appl. No.: 315,987
Filed: Sept. 30, 1994
Primary Examiner: Fleisher; Mindy
Assistant Examiner: Degen; Nancy J.

Abstract

The invention provides methods for the identification and discovery of agents which are inhibitors and activators of RNA and DNA-dependent nucleic acid polymerases. The essential feature of the invention is the incorporation of a functional polymerase binding site sequence (PBS) into a nucleic acid molecule which is chosen for its ability to confer a discernible characteristic via its sequence specific activity such that the incorporation of the PBS renders the nucleic acid molecule a functional template for a predetermined RNA or DNA-template directed nucleic acid polymerase (1). In the presence of the polymerase, suitable primer molecules, and any necessary accessory molecules, catalytic extension of the strand of nucleic acids complementary to the template occurs, resulting in a partial or total elimination of (or increase in) the characteristic conferring activity of the reporter-template molecule described due to the antisense effects of the complementary strand or other polymerase-mediated effects. Candidate inhibitors and activators are evaluated for their specific effects on polymerase function, versus polymerase-unrelated effects on reporter-template function, by comparing their ability to decrease or increase the extent of the characteristic conferred by the activity of the reporter-template molecule in the assay to their ability to do so in the control situation in which the activity of the polymerase has been eliminated from the assay. A novel method for controlling gene expression and, in general, the activity of a nucleic acid molecule is also disclosed.

26 Claims, 4 Drawing Figures

US PAT NO: 5,583,016
DATE ISSUED: Dec. 10, 1996
TITLE: Mammalian **telomerase**
INVENTOR: Bryant Villeponteau, San Carlos, CA
Junli Feng, San Carlos, CA
Walter Funk, Union City, CA
William H. Andrews, Richmond, CA
ASSIGNEE: Geron Corporation, Menlo Park, CA (U.S. corp.)
APPL-NO: 08/330,123
DATE FILED: Oct. 27, 1994
ART-UNIT: 184
PRIM-EXMR: Robert A. Wax
ASST-EXMR: Gabriele E. Bugaisky
LEGAL-REP: Kevin R. Kaster, William M. Smith, John R. Storella

ABSTRACT:
Nucleic acids comprising the RNA component of a mammalian **telomerase** are useful as pharmaceutical, therapeutic, and diagnostic reagents.

US PAT NO: 5,489,508
DATE ISSUED: Feb. 6, 1996
TITLE: Therapy and diagnosis of conditions related to telomere length and/or **telomerase** activity
INVENTOR: Michael D. West, Belmont, CA
Jerry Shay, Dallas, TX
Woodring Wright, Arlington, TX

ASSIGNEE: University of Texas System Board of Regents, Austin, TX (U.S. corp.)
APPL-NO: 08/038,766
DATE FILED: Mar. 24, 1993
ART-UNIT: 187
PRIM-EXMR: W. Gary Jones
ASST-EXMR: Carla Myers
LEGAL-REP: Richard Warburg, Kevin Kaster, Amy Stark

ABSTRACT:
Method and compositions are provided for the determination of telomere length and **telomerase** activity, as well as the ability to inhibit **telomerase** activity in the treatment of proliferative diseases. Particularly, primers are elongated under conditions which minimize interference from other genomic sequences, so as to obtain accurate determinations of telomeric length or **telomerase** activity. In addition, compositions are provided for intracellular inhibition of **telomerase** activity.

ACCESSION NUMBER: 1997:436143 CAPLUS
DOCUMENT NUMBER: 127:47064
TITLE: **Preparation** of **human telomerase** for diagnosis of tumors
INVENTOR(S): Murofushi, Kimiko
PATENT ASSIGNEE(S): Soosei K. K., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 5 pp.
CODEN: JKXXAF

	NUMBER	DATE	
PATENT INFORMATION:	JP 09154575 A2	970617	Heisei
APPLICATION INFORMATION:	JP 96-282948	961004	
PRIORITY APPLN. INFO.:	JP 95-284559	951004	

DOCUMENT TYPE: Patent
LANGUAGE: Japanese

AB Telomerase useful in the diagnosis of tumors is prepd. from the Namalwa cell homogenate. After a few steps of chromatog., the enzyme was purified with the (TTAGGG)2-contg. affinity chromatog. The enzyme exhibits a mol. wt. 300 kDa by gel filtration. The enzyme is predicted by SDS-PAGE (reducing) to contains a 140-, an 80-, and a 50-kDa components.

ACCESSION NUMBER: 96-239169 [24] WPINDEX
DOC. NO. NON-CPI: N96-200226
DOC. NO. CPI: C96-076272
TITLE: Novel telomerase associated polypeptide(s) and related nucleic acid - useful for detecting e.g. tumour cells or pathogens.
DERWENT CLASS: B04 D16 S03
INVENTOR(S): GOTTSCHLING, D E; SINGER, M S
PATENT ASSIGNEE(S): (ARCH-N) ARCH DEV CORP
COUNTRY COUNT: 62
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9612811	A2	960502	(9624)*	EN	349

RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE

SZ UG
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS
JP KE KG KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO
RU SD SE SG SI SK TJ TM TT UA UG UZ VN
AU 9642786 A 960515 (9634)
WO 9612811 A3 960801 (9641)

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9612811	A2	WO 95-US13801	951020
AU 9642786	A	AU 96-42786	951020
WO 9612811	A3	WO 95-US13801	951020

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9642786	A	Based on	WO 9612811

PRIORITY APPLN. INFO: US 95-431080 950428; US 94-326781 941020

AB WO 9612811 A UPAB: 960618
A novel nucleic acid (NA) segment comprises: (a) a NA segment comprising a region of more than 17 contiguous bases with the same sequence as or complementary to 17 contiguous bases of the 1301, 2434, 2117, 1882, 1094 or 807 bp sequences given in the specification; or (b) a NA segment of 17-10,000 bases which hybridises to these sequences, or their complements, under standard hybridisation conditions.

USE - The NA segment can detect a non-ciliate **telomerase**-associated gene in a sample (claimed), e.g. those suspected on contg. tumour cells, a pathogen or a sperm or egg cell. The segment can also express the polypeptides, and can be used in NA hybridisation assays and in the prepn. of primers. The substance identified can modify a cell's replicative capacity (claimed), e.g. inhibit tumour or pathogenic cell replication, or promote sperm or egg cell replication, by modifying **telomerase** activity. The transformed cells, partic. **human** cells, are useful in the development of diagnostics and therapeutics. The NAs, polypeptides and **antibodies** are useful in molecular biology and immunodetection kits to detect **telomerase** -associated components. These are useful to diagnose conditions associated with infertility. The affinity column can be used to purify eukaryotic **telomerase** complexes.
Dwg.0/8

ACCESSION NUMBER: 97-099928 [09] WPINDEX
DOC. NO. CPI: C97-031908
TITLE: DNA encoding essential RNA components of human **telomerase** - also truncated or recombinant **telomerase**, useful for diagnosis and treatment of cancer and infection by eukaryotic parasites.
DERWENT CLASS: **B04** C07 D16
INVENTOR(S): AUTEXIER, C; GREIDER, C
PATENT ASSIGNEE(S): (COLD-N) COLD SPRING HARBOR LAB
COUNTRY COUNT: 22
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9640868	A1	961219	(9709)*	EN	48
RW: AT BE CH DE DK ES FI FR GB GR IE IT LU MC NL PT SE					
W: AU CA JP MX US					
AU 9661022	A	961230	(9716)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9640868	A1	WO 96-US9517	960606
AU 9661022	A	AU 96-61022	960606

FILING DETAILS:

PATENT NO	KIND		PATENT NO
AU 9661022	A	Based on	WO 9640868

PRIORITY APPLN. INFO: US 95-478352 950607

AB WO 9640868 A UPAB: 970228

The following isolated sequences (I) of human **telomerase** (hTR) are new: (a) nucleotides (nt) 44-204; (b) nt 1-203, 1-273 or 1-418; (c) nt 44-204 and sequential deoxynucleotides but shorter than 1-445.

USE - The new RNA and DNA is used, in hybridisation assays, to detect or quantify **telomerase activity** in cells, tissue or fluid samples, e.g. for diagnosis of eukaryotic parasites (yeast and protozoa) or tumours. It is also useful as primers for amplification assays. The truncated or recombinant VT is used therapeutically to increase **telomerase activity** (also as reagents in the screening assay) while (II) or other **inhibitors** such as antisense molecules, are used to **reduce** such **activity**. Typical applications are initiation/restoration of **activity** to cause senescence or to prevent immortalisation of cells in tumours or parasites. (I) are also used to produce recombinant **telomerase**, which can then be used conventionally to raise antibodies for diagnostic detection of **telomerase**.

ADVANTAGE - Detecting **telomerase** allows early diagnosis of tumour or infection, before clinical signs are manifest. **Telomerase inhibitors** directed against e.g. Trypanosoma should cause fewer side effects than drugs currently used to treat such infections. (I) encodes those parts of hTR RNA essential for **activity** but are significantly shorter than the endogenous RNA component.

Dwg.0/7

ACCESSION NUMBER:	96-309594 [31] WPINDEX
DOC. NO. NON-CPI:	N96-260073
DOC. NO. CPI:	C96-098972
TITLE:	**Telomerase** protein and related DNA, antibodies, transgenic cells, etc. - for diagnosis and treatment of cancer and infection by eukaryotic microbes, also new **telomerase** inhibitors.
DERWENT CLASS:	**B04** D16 P14 S03
INVENTOR(S):	AUTEXIER, C; COLLINS, K; GREIDER, C; HEMISH, J M; KOBAYASHI, R; YANG, X H

PATENT ASSIGNEE(S): (COLD-N) COLD SPRING HARBOR LAB
COUNTRY COUNT: 20
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9619580	A2	960627	(9631)*	EN	56
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE					
W: CA JP MX US					
WO 9619580	A3	960829	(9643)		

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9619580	A2	WO 95-US16531	951218
WO 9619580	A3	WO 95-US16531	951218

PRIORITY APPLN. INFO: US 94-359125 941219

AB WO 9619580 A UPAB: 960808
A pure **telomerase** protein component (A) is new. Also new are; (1) DNA (I) encoding (A); (2) anti-**telomerase** antibody (Ab) that binds to one of the peptides AEGYSDINVRG, QNEFQFNNVK or EFGLEPNILT, or to all or part of (A); (3) method for identifying a cpd. (C) that **inhibits**, destroys or interferes with **telomerase activity** in eukaryotic cells; and (4) transgenic eukaryotic or prokaryotic cells or eukaryotic organism contg. (I) or its complement.

USE - Ab are immunoassay reagents to detect (A), esp. to identify immortalised cells, or predisposition to immortalisation, partic. cancer, or to diagnose disease caused by a eukaryotic microbe (all claimed). Inhibitors of (A) and (A) itself can be used for therapy or diagnosis, esp. inhibitors are used to treat infection by fungi and protozoa (claimed). (I) is used to produce recombinant (A) (claimed) or to isolate similar genes from other organisms, esp. humans, while transformed cells can be used in gene therapy.

ADVANTAGE - Since somatic cells do not generally require **telomerase**, inhibitors of (A) should have little or no toxicity to the host.

Dwg.0/11

ACCESSION NUMBER: 95:1782515 PATOSEP ED 971019 EW 9741 FS OS
TITLE: **TELOMERASE** PROTEIN COMPONENT.
TELOMERASE PROTEIN-KOMPONENTE.
FRACTION PROTEINIQUE DE LA **TELOMERASE**.
INVENTOR(S): GREIDER, Carol, 87 Bay Drive East, Huntington, NY 11743, US;
COLLINS, Kathleen, 111 Prime Avenue, Huntington, NY 11743, US;
KOBAYASHI, Ryuji, 1152 Cove Edge Road, Syosset, NY 11791, US;
YANG, Xiaohong, Helena, 90 Cuba Hill Road, Greenlawn, NY 11740, US;
HEMISH, Jill, M., 4 Fenwood Road, Huntington Station, NY 11746, US;
AUTEXIER, Chantal, 188 Vernon Valley Road, East Northport, NY 11731, US
PATENT ASSIGNEE(S): COLD SPRING HARBOR LABORATORY, 100 Bungtown Road,

	Cold Spring Harbor, NY 11724, US
PATENT ASSIGNEE NO:	705233
AGENT:	Holdcroft, James Gerald, Dr. et al, Graham Watt & Co. 3 Gray's Inn Square, London WC1R 5AH, GB
AGENT NUMBER:	31912
SOURCE:	Wila-EPZ-1997-H41-T1a
DOCUMENT TYPE:	Patent
LANGUAGE:	Anmeldung in Englisch; Veroeffentlichung in Englisch
DESIGNATED STATES:	R AT; R BE; R CH; R DE; R DK; R ES; R FR; R GB; R GR; R IE; R IT; R LI; R LU; R MC; R NL; R PT; R SE
PATENT INFO.PUB.TYPE:	EPA2 EUROPAEISCHE PATENTANMELDUNG (Internationale Anmeldung)

PATENT INFORMATION:

PATENT NO	KIND	DATE	
EP 799315	A2	971008	
'OFFENLEGUNGS' DATE:		971008	
APPLICATION INFO.:	EP 95-943470	951218	
PRIORITY APPLN. INFO.:	US 94-359125	941219	
RELATED DOC. INFO.:	WO 95-US16531	951218	INTAKZ
	WO 9619580	960627	INTPNR

NOTE: ABSTRACT in PATOSWO

EPA2 EUROPAEISCHE PATENTANMELDUNG (Internationale Anmeldung)
EPLU LEGAL STATUS, UPDATE

ATTACHMENT B

1. *Agents that target telomerase and telomeres. Raymond, E., Sun, D., Chen, Shih-Fong., Windle, B ane Von Hoff, D.D. Curr. Opin. Biotechnol. 7: 583-91, 1996.

2. *TLP1: A gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. Nakayama, J., Saito, M., Nakamura, H., Matsuura, A. and Ishikawa, F. Cell 88: 875-84, 1997.

3. *A mammalian telomerase-associated protein. Harrington, L., McPhail, T., Mar, V., Zhou, W., Oulton, R., Amgen EST program, Bass, M.B., Arruda, I. and Robinson, M.O. Science 275: 973-977, 1997.

4. *Telomeres, telomerase, and immortality. Rhyu, M.S. J. Natl. Cancer Inst. 87: 884-894, 1995.

5. ^Purification of tetrahymena telomerase and cloning of genes encoding the two protein components of the enzyme. Collins, K., Kobayashi, R., and Greider, C.W. Cell 81: 677-86, 1995.

6. *Telomere dynamics and telomerase activation in tumor progression: prospects for prognosis and therapy. Healy, K.C. Oncol. Res. 7: 121-130, 1995.

7. *Telomeres, telomerase and senescence. Greider, C.W. BioEssays 12: 363-369, 1990.

8. ^Telomeres: beginning to understand the end. Zakian, V.A. Science 270: 1601-7, 1995.

9. +Telomerase in development and differentiation. Hiyama, E., Hiyama, K., Tatsumoto, N. and Yokoyama, T. Siashin Igaku 51: 2255-2259, 1996.

10. +Telomerase and cancer. Hiyama, K. and Hiyama, E. Bio. Ind. 13: 5-13, 1996.

11. +Linking telomerase and tumors. Genesis Report-Dx, vol. 4, no. 6. 1995. Publisher Genesis Group Associates.

12. +Cancer genetics gene regulates telomerase resulting in death of cancer cells. Gene Therapy Weekly, 11 September 1995. Publisher Charles W. Henderson.

* Copy of article is enclosed.
+ Article has not been reviewed.
^ Article reviewed, no copy needed.

* Copy of article is enclosed.

1.
***TI - Agents that target telomerase and telomeres.**
SO - Curr Opin Biotechnol 1996 Dec;7(6):583-91
AU - Raymond E; Sun D; Chen SF; Windle B; Von Hoff DD
AD - Human Telomerase Research Group, Institute for Drug Development - Cancer Therapy and Research Center, 14960 Omicron Drive, San Antonio, TX 78245-3217, USA.
MJ - Antineoplastic Agents [chemistry]; Drug Design; Enzyme Inhibitors [pharmacology]; Telomerase [antagonists & inhibitors]; Telomere [drug effects]
MN - Antineoplastic Agents [pharmacology]; Drug Resistance, Neoplasm; Enzyme Inhibitors [chemistry]; Mice; Models, Molecular; Neoplasms [drug therapy] [genetics]; Nucleic Acid Conformation; Nucleoproteins [drug effects]; Reverse Transcriptase Inhibitors [chemistry] [pharmacology]; Telomerase [genetics] [metabolism]; Telomere [chemistry] [genetics]
MT - Animal; Human; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
PT - JOURNAL ARTICLE; REVIEW (91 references); REVIEW, TUTORIAL
AB - Telomeres are guanine-rich regions that are located at the ends of chromosomes and are essential for preventing aberrant recombination and protecting against exonucleolytic DNA degradation. Telomeres are maintained by telomerase, an RNA-dependent DNA polymerase. Because telomerase is known to be expressed in tumor cells, which concurrently have short telomeres, and not in most somatic cells, which usually have long telomeres, telomerase and telomere structures have been recently proposed as attractive targets for the discovery of new anticancer agents. The most exciting current strategies are aimed at specifically designing new drugs that target telomerase or telomeres and new models have been formulated to study the biological effects of inhibitors of telomerase and telomeres both in vitro and in vivo.
LA - English
RN - EC 2.7.7.- (Telomerase); 0 (Antineoplastic Agents); 0 (Enzyme Inhibitors); 0 (Nucleoproteins); 0 (Reverse Transcriptase Inhibitors)

2.
***TI - TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family.**
SO - Cell 1997 Mar 21;88(6):875-84
AU - Nakayama J; Saito M; Nakamura H; Matsuura A; Ishikawa F
AD - Department of Life Science, Tokyo Institute of Technology, Yokohama, Japan.
MJ - Repetitive Sequences, Nucleic Acid; Telomerase [genetics]
MN - Blotting, Northern; Blotting, Western; COS Cells [physiology]; Cloning, Molecular; DNA, Complementary [analysis]; Gene Expression Regulation, Enzymologic [physiology]; Mammals; Mice; Molecular Sequence Data; Precipitin Tests; Rats; Sequence Homology, Amino Acid
MT - Animal; Human; Support, Non-U.S. Gov't
PT - JOURNAL ARTICLE
AB - We have cloned and characterized the rat telomerase protein component 1 gene (TLP1), which is related to the gene for Tetrahymena p80. The cDNA encodes a 2629 amino acid sequence and produces the TLP1 proteins p240 and p230. The anti-TLP1 antibody specifically immunoprecipitated the telomerase activity. Moreover, p240 and p230 were copurified with telomerase activity in a series of extensive purification experiments. These results strongly suggest that the TLP1 proteins are components of, or are closely associated with, the

rat telomerase. A pulse-chase experiment showed that p240 is modified to p230 in vivo. p230 was the dominant form in telomerase-positive cells, suggesting that modification of the TLP1 protein may regulate telomerase activity in vivo.
LA - English
RN - EC 2.7.7.- (Telomerase); 0 (DNA, Complementary)

3.
***TI - A mammalian telomerase-associated protein [see comments]**
CM - Comment in: Science 1997 Feb 14; 275(5302):928
SO - Science 1997 Feb 14;275(5302):973-7
AU - Harrington L; McPhail T; Mar V; Zhou W; Oulton R; Bass MB; Arruda I; Robinson MO
AD - Arruda, Ontario Cancer Institute-Amgen Institute, Department of Medical Biophysics, University of Toronto, 620 University Avenue, Toronto, Ontario M5G 2C1, Canada.
MJ - Carrier Proteins [chemistry] [metabolism]; RNA [metabolism]; Telomerase [chemistry]
MN - Amino Acid Sequence; Blotting, Northern; Carrier Proteins [genetics] [immunology]; Cell Line; Cloning, Molecular; DNA, Complementary [genetics]; Mice; Molecular Sequence Data; Precipitin Tests; RNA, Messenger [genetics] [metabolism]; Sequence Homology, Amino Acid; Telomerase [genetics] [metabolism]; Tetrahymena [chemistry] [genetics]; Transfection; Tumor Cells, Cultured
MT - Animal; Human
PT - JOURNAL ARTICLE
AB - The telomerase ribonucleoprotein catalyzes the addition of new telomeres onto chromosome ends. A gene encoding a mammalian telomerase homolog called TP1 (telomerase-associated protein 1) was identified and cloned. TP1 exhibited extensive amino acid similarity to the Tetrahymena telomerase protein p80 and was shown to interact specifically with mammalian telomerase RNA. Antiserum to TP1 immunoprecipitated telomerase activity from cell extracts, suggesting that TP1 is associated with telomerase in vivo. The identification of TP1 suggests that telomerase-associated proteins are conserved from ciliates to humans.
LA - English
RN - EC 2.7.7.- (Telomerase); 0 (telomerase-associated protein 1); 0 (Carrier Proteins); 0 (DNA, Complementary); 0 (RNA, Messenger); 63231-63-0 (RNA)
SI - GENBANK/U86136; GENBANK/U86137

4.
***TI - Telomeres, telomerase, and immortality [see comments]**
CM - Comment in: J Natl Cancer Inst 1995 Jun 21; 87(12):859-61
SO - J Natl Cancer Inst 1995 Jun 21;87(12):884-94
AU - Rhyu MS
AD - National Cancer Institute, Bethesda, MD, USA.
MJ - DNA Nucleotidylexotransferase [metabolism]; Neoplasms [enzymology]; Telomere [enzymology]
MN - Apoptosis [physiology]; Base Sequence; Cell Survival [physiology]; DNA Nucleotidylexotransferase [antagonists & inhibitors] [genetics]; Enzyme Activation [physiology]; Mitosis [physiology]; Molecular Sequence Data; Telomere [genetics]
MT - Animal; Human
PT - JOURNAL ARTICLE; REVIEW (57 references); REVIEW, TUTORIAL

AB - A current hypothesis gaining prominence proposes that activation of the enzyme telomerase is necessary for cells to become immortal, or capable of proliferating indefinitely. The theory suggests that almost all cancer cells must attain immortality for progression to malignant states and, hence, require activation of telomerase. This article reviews the function and formation of telomeres as background to evaluating the "telomere hypothesis." Experiments in support of and experiments that challenge the hypothesis are examined. Possible approaches to telomerase inhibition are discussed.
LA - English
RN - EC 2.7.7.31 (DNA Nucleotidylexotransferase)

5.
TI - Purification of Tetrahymena telomerase and cloning of genes encoding the two protein components of the enzyme.
SO - Cell 1995 Jun 2;81(5):677-86
AU - Collins K; Kobayashi R; Greider CW
AD - Cold Spring Harbor Laboratory, New York 11724, USA.
MJ - DNA Nucleotidylexotransferase [genetics]; Genes, Protozoan [genetics]; Protozoan Proteins [genetics]; Ribonucleoproteins [genetics]; Tetrahymena thermophila [genetics]
MN - Amino Acid Sequence; Antibodies, Protozoan; Base Sequence; Cloning, Molecular; DNA Nucleotidylexotransferase [immunology]; DNA, Complementary [genetics]; Electrophoresis, Polyacrylamide Gel; Immunoblotting; Molecular Sequence Data; Nucleic Acid Conformation; Precipitin Tests; Protein Conformation; RNA, Protozoan [metabolism]; Ribonucleoproteins [isolation & purification]; Sequence Analysis; Tetrahymena thermophila [enzymology] [isolation & purification]
MT - Animal; Support, Non-U.S. Gov't; Support, U.S. Gov't, P.H.S.
PT - JOURNAL ARTICLE
AB - Telomerase is a ribonucleoprotein DNA polymerase that catalyzes the de novo synthesis of telomeric simple sequence repeats. We describe the purification of telomerase and the cloning of cDNAs encoding two protein subunits from the ciliate Tetrahymena. Two proteins of 80 and 95 kDa copurified and coimmunoprecipitated with telomerase activity and the previously identified Tetrahymena telomerase RNA. The p95 subunit specifically cross-linked to a radiolabeled telomeric DNA primer, while the p80 subunit specifically bound to radiolabeled telomerase RNA. At the primary sequence level, the two telomerase proteins share only limited homologies with other polymerases and polymerase accessory factors.
LA - English
RN - EC 2.7.7.31 (DNA Nucleotidylexotransferase); 0 (Antibodies, Protozoan); 0 (DNA, Complementary); 0 (Protozoan Proteins); 0 (Ribonucleoproteins); 0 (RNA, Protozoan)
SI - GENBANK/U25641; GENBANK/U25642

6.
***TI - Telomere dynamics and telomerase activation in tumor progression:** prospects for prognosis and therapy.
SO - Oncol Res 1995;7(3-4):121-30
AU - Healy KC
AD - Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510, USA.
MJ - Neoplasms [enzymology] [pathology]; Telomerase [metabolism]; Telomere [physiology]

MN - Base Sequence; Disease Progression; Enzyme Activation; Neoplasms [therapy]; Prognosis; Repetitive Sequences, Nucleic Acid
MT - Human
PT - JOURNAL ARTICLE; REVIEW (84 references); REVIEW, TUTORIAL
AB - Eukaryotic telomeres provide a reservoir of redundancy to compensate for incomplete replication of chromosome ends. In multicellular eukaryotes, they are eroded by a varying number of base pairs at every cell division. When telomere repeats are critically shortened, DNA damage response pathways involving p53 (and in some cell types retinoblastoma protein) are invoked, leading to "M1 senescence" in normal cells; cancer cells, which frequently lack normal p53 and RB functions, often develop chromosomal instability leading to telomeric associations, ring chromosomes, and breakage-fusion-bridge cycles. These consequences of telomere erosion exert selection pressure for activation of the ribonucleoprotein enzyme telomerase, which adds new telomeric repeats at chromosome ends, and in vertebrates normally is active only in the germ line and the early embryo. Somatic cells that reactivate telomerase in vitro or in vivo become immortal. Telomerase activity has been found in many advanced and metastatic human cancers, suggesting that telomerase-dependent M2 immortalization may contribute to metastatic potential. When mammalian telomerases are isolated and their genes cloned and sequenced, the localization of telomerase expression in tumors may provide prognostic indicators of metastatic potential. The abrogation of telomerase function by pharmacological inhibition, genetic disruption, or repression of gene expression is a potential avenue of antimetastatic therapy.
LA - English
RN - EC 2.7.7.- (Telomerase)

7.
***TI - Telomeres, telomerase and Senescence.**
SO - BioEssays 1990 Aug. 12(8):363-369
AU - Greider CW;
MT - Human
PT - JOURNAL ARTICLE
LA - English
RN - EC 2.7.7.- (Telomerase)

8.
TI - Telomeres: beginning to understand the end.
SO - Science 1995 Dec 8;270(5242):1601-7
AU - Zakian VA
AD - Department of Molecular Biology, Princeton University, NJ 08544, USA.
MJ - Telomere [physiology]
MN - Base Sequence; Cell Cycle; Chromosomes [metabolism] [physiology]; DNA Replication; DNA-Binding Proteins [metabolism]; DNA [analysis] [chemistry] [metabolism]; Gene Expression Regulation; Molecular Sequence Data; Telomerase [metabolism]; Telomere [chemistry]
MT - Animal; Human; Support, U.S. Gov't, P.H.S.
PT - JOURNAL ARTICLE; REVIEW (147 references); REVIEW, TUTORIAL
AB - Telomeres are the protein-DNA structures at the ends of eukaryotic chromosomes. In yeast, and probably most other eukaryotes, telomeres are essential. They allow the cell to distinguish intact from broken chromosomes, protect chromosomes from degradation, and are substrates for novel replication mechanisms. Telomeres are usually replicated by telomerase, a telomere-specific reverse transcriptase, although telomerase-independent mechanisms of

telomere maintenance exist. Telomere replication is both cell cycle- and developmentally regulated, and its control is likely to be complex. Because telomere loss causes the kinds of chromosomal changes associated with cancer and aging, an understanding of telomere biology has medical relevance.
LA - English
RN - EC 2.7.7.- (Telomerase); 0 (DNA-Binding Proteins); 9007-49-2 (DNA)

9.
ACCESSION NUMBER: 1996:686981 CAPLUS
DOCUMENT NUMBER: 126:73120
TITLE: **Telomerase** in development and differentiation
AUTHOR(S): Hiyama, Eiso; Hiyama, Keiko; Tatsumoto, Naokuni; Yokoyama, Takashi
CORPORATE SOURCE: Sch. Med., Hiroshima Univ., Hiroshima, 734, Japan
SOURCE: Saishin Igaku (1996), 51(11), 2255-2259
CODEN: SAIGAK; ISSN: 0370-8241
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 20 refs., on **telomerase activity** in normal human embryonic development and cell differentiation and in neuroblastoma, and significance of **telomerase** in the **diagnosis** and treatment of tumors.

10.
ACCESSION NUMBER: 1996:234145 CAPLUS
DOCUMENT NUMBER: 124:285539
TITLE: **Telomerase** and cancer
AUTHOR(S): Hiyama, Keiko; Hiyama, Eiso
CORPORATE SOURCE: Fac. of Medicine, Hiroshima Univ., Japan
SOURCE: Bio Ind. (1996), 13(3), 5-13
CODEN: BIINEG; ISSN: 0910-6545
DOCUMENT TYPE: Journal; General Review
LANGUAGE: Japanese
AB A review with 32 refs., on structure and function of telomere DNA, **telomerase**, two step model of cell aging and telomere theory, telomere length and **telomerase activity** in human cancer, and **telomerase** as target for novel anti-tumor therapy and **diagnosis**.

11.
ACCESSION NUMBER: 95:91307 NLDB
TITLE: Linking **Telomerase** and Tumors
SOURCE: Genesis Report-Dx, (1 May 1995) Vol. 4, No. 6.
ISSN: 1061-2289.
PUBLISHER: Genesis Group Associates, Inc
DOCUMENT TYPE: Newsletter
LANGUAGE: English
WORD COUNT: 407
TI Linking **Telomerase** and Tumors
TX Other studies by Shay found **telomerase activity** in about 85% of a wide variety of cancers, in 98 of 100 panels of

human immortalized cell lines, and in 90 of 101 tumor biopsies. However, Shay did not identify **telomerase** expression in 22 normal cells or in 50 normal tissues. He also reported that the senescence induced by short telomeres in fibroblasts was mediated by two known tumor suppressor genes, p53 and pRb.

Shay commented, "These findings suggest that continued proliferation of most tumor cells is dependent upon the activation of **telomerase**. Therefore, tests for **telomerase** **activity** in primary tumors should be useful both for **diagnostic** and prognostic purposes. In our initial studies, only 25% of axillary lymph node-negative breast tumors expressed **telomerase activity**. In contrast, nearly all lymph node-positive tumors were **telomerase** positive. The presence of **telomerase activity** in node-negative tumors could potentially indicate a higher probability of cancer recurrence.

"Development of anticancer agents based on **telomerase** may be highly efficacious. Normal somatic cells do not express **telomerase**, so it would be predicted that this type of agent would also possess great specificity, low toxicity, and reduced side effects." An inhibitor would probably be used as adjunctive **therapy**, however, because Shay noted that the tumor cells would stop proliferating only "after the number of doublings related to the telomere length at the time of treatment."

Calvin Harley, PhD, vice president of research at Geron, said researchers at Geron and their academic collaborators had cloned human **telomerase** RNA and found more **activity** in early progenitor hematopoietic cells than in more differentiated types. He said he hopes to use his own polymerase chain reaction (PCR) assay for **telomerase** - a test he calls the Telomere PCR Amplification Protocol - and the human clone for the RNA to study further the differentiation of stem cells into somatic cell types. In a collaboration, he also hopes to use the RNA "as a handle to obtain the protein clones for the enzyme, examine its biochemistry and its moiety of **telomerase** further, and ultimately to have a fairly aggressive drug development and discovery program in order to apply the knowledge we've gained to treat age-related diseases, including cancers." A number of companies are known to be developing **diagnostics** of **telomerase activity** and researching drugs to **inhibit** its **activity**, but Harley claimed that Geron is the only company devoted primarily to this research.

12.

ACCESSION NUMBER:	97:109534 NLDB
TITLE:	Cancer Genetics Gene Regulates **Telomerase**, Resulting in Death of Cancer Cells
SOURCE:	Gene Therapy Weekly, (11 Sep 1995) . ISSN: 1078-2842.
PUBLISHER:	Charles W Henderson
DOCUMENT TYPE:	Newsletter

LANGUAGE: English
WORD COUNT: 430

TX Research describing the first cloning of the RNA component of human **telomerase** showed that **telomerase** inhibition leads to cancer cell death, according to a study published in the September 1, 1995, issue of Science by researchers from Geron Corporation, Menlo Park, California, and Cold Spring Harbor Laboratory (CSHL).

Telomerase is believed to be an "immortalizing enzyme" which gives cancer cells an infinite capacity to replicate. It could serve as a single, specific target for anti-cancer **therapeutics**. Geron research has shown **telomerase** is found in high levels in cancer cells, but is inactive in nearly all normal cells, demonstrating a strong link between **telomerase** levels and cell "immortality" in cancer.

In the study reported, researchers using immortalized kidney cells successfully cloned human **telomerase** RNA (hTR) and subsequently found that these cells contained elevated hTR levels. When the cloned gene was used to inhibit **telomerase**, proliferation of human cancer cells was likewise inhibited after a short period of growth and tumor cell death resulted.

The senior author of the article was Bryant Villeponteau, Ph.D., senior staff scientist at Geron. Carol Greider, Ph.D., senior staff investigator, led the team of collaborators at CSHL. In the same issue of Science, the Geron and CSHL team also report on the cloning and characterization of the mouse **telomerase** gene. This will provide a key experimental tool for further research.

"These results indicate that human **telomerase** is a critical enzyme for the growth and proliferation of immortal tumor cells," said Calvin B. Harley, Ph.D., Geron's vice president of research. "We now have demonstrated that an inhibitor of **telomerase** has potential as a specific and effective **therapeutic** against human cancer."

In cancer, activation of the **telomerase** enzyme apparently stops the molecular clock of aging by maintaining the length of telomeres, allowing cancer cells to proliferate indefinitely. Geron believes that drugs that specifically inhibit the **telomerase** enzyme will cause cancer cells to die prematurely. Since **telomerase** has been found in most types of cancer tumors - and not in most healthy tissues Geron scientists believe **telomerase** may be a specific, anti-cancer target for nearly all forms of cancer.

Prior Art Searches, Inc.
Ted Apple, Esquire
HUMAN TELOMERASE
November 18, 1997

ATTACHMENT C

R29GM49157 SHIPPEN, DOROTHY TELOMERASE RIBONUCLEOPROTEIN STRUCTUR
-PROJECT NUMBER.....5 R29 GM49157-03
INSTITUTEGM FY 95 SHIPPEN, DOROTHY
INITIAL REVIEW GROUP IRGBIO TEXAS A&M UNIVERSITY
AWARD AMOUNT..... $96,335
COLLEGE STATION, TX 77843
PERFORMING ORGANIZATION: TEXAS AGRICULTURAL EXPERIMENT STATION
TITLE TELOMERASE RIBONUCLEOPROTEIN STRUCTURE
FUTURE YEARS 2
ABSTRACT:

Telomeres are the specialized DNA-protein structures that cap the ends of eukaryotic chromosomes and serve an essential role in maintaining chromosome stability and proper nuclear architecture. Highly conserved throughout evolution, telomeric DNA consists of simple tandemly repeated sequences. A novel ribonucleoprotein (RNP) enzyme called telomerase is responsible for synthesizing telomeric DNA. Within the telomerase RNA moiety is a sequence complementary to the telomeric repeats and this domain serves as the template for DNA synthesis. Thus, telomerase is a specialized type of reverse transcriptase. The long range goal of this project is to elucidate the mechanism of telomere synthesis by the telomerase enzyme. Currently, our understanding of this process is impeded by the lack of information concerning the components of the telomerase RNP. Although both RNA and protein are required for activity, the telomerase RNA has only been partially characterized, while the protein component(s) of this enzyme have not yet been identified. This application is aimed at further analysis of telomerase RNP structure by exploiting the component we have in hand, the RNA moiety. The experimental system chosen for these studies are hypotrichous ciliates, Euplotes and Oxytricha, two of the richest known sources of telomeres. There are two major goals in this proposal. The first is an examination of the telomerase RNA, analyzing the secondary structure of this molecule by phylogenetic and in vitro experimental approaches. In addition, we will also explore the function of the telomeric templating domain in the Oxytricha telomerase RNA. The second part of this proposal investigates RNA-protein interactions within the telomerase RNP. Two different approaches based on techniques developed for the analysis of other small nuclear RNP complexes will be used to identify proteins that are lightly associated with the telomerase RNA. The first will be to partially purify the Euplotes telomerase RNP using conventional column chromatography and equilibrium gradient centrifugation, following either enzymatic activity or the telomerase RNA component as a marker for the telomerase complex. Samples will be further purified by affinity selection of Euplotes telomerase RNP particles using oligonucleotides complementary to the telomerase RNA moiety. A second strategy to identify polypeptides associated with the telomerase RNA will assay for the assembly of proteins from partially purified telomerase preparations onto exogenous telomerase RNA transcripts in vitro. Proteins that bind to the telomerase RNA will be identified and further characterized.

U19CA677600003 WINDLE, BRADFORD TELOMERE AND TELOMERASE INTERACTIVE A

-PROJECT NUMBER.....5 U19 CA67760-02 SUB: 0003
INSTITUTECA FY 96 WINDLE, BRADFORD
INITIAL REVIEW GROUP IRGSRC UNIV OF TEXAS HLTH SCI CTR
AWARD AMOUNT..... 7703 FLOYD CURL DR
SAN ANTONIO, TX 78284-7862
PERFORMING ORGANIZATION: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANT
TITLE TELOMERE AND TELOMERASE INTERACTIVE AGENTS
SUB TITLE TELOMERASES AND TELOMERASE MOLECULAR BIOLOGY
FUTURE YEARS 3
ABSTRACT:

Telomeres have a G-rich repeated DNA sequence that maintains chromosome end stability. Telomerase is a reverse transcriptase-like ribonucleoprotein that maintains the telomere sequence. Telomerase is expressed in the majority of tumor cells but not in normal cells. Telomerase and telomeres are ideal tumor-specific targets for anti-cancer therapeutic strategies. Our preliminary studies show the discovery of the first telomerase inhibitors. Our studies also show that telomerase inhibitors induce telomere shortening, chromosome end fusion, and eventually cell death. Cells have shown the ability to adapt to telomerase inhibition.

The goals of this proposal are to l) elucidate the molecular structure of telomerase and provide a more defined target for our anti-cancer strategies, and 2) determine the mechanism(s) of cellular adaptation to telomerase inhibitors. These goals will be accomplished by mapping and cloning the genes for the telomerase proteins and RNA template. Through our collaboration with Program l, purified telomerase will be used as a source of purified telomerase RNA for cloning. Somatic cell genetics will be used to map the telomerase activity. The new technique of Differential Display will be used to detect mRNAs expressed in posttelomerase-activated cells that are not expressed in pre-telomerase activated cells. The mapping will be coordinated with the isolation of candidate cDNA clones for the proteins and RNA to accelerate the identification of legitimate clones. The cDNA clones of the RNA will be screened for appropriate template sequence, secondary structure, and ability to direct in vitro and in vivo telomerase activity. The cDNAs for telomerase proteins will be expressed for production of antibodies.

The antibodies and DNA clones will serve as probes for use in studies of telomerase expression in Program 3 and in tumor specimens from Program 4. The data from these studies will be used by Program 2 for structure modeling and specific drug design. Potential telomerase inhibitors, such as anti RNA oligonucleotides will be studied by Program l. The mechanism of adaptation to telomerase inhibitors will also be studied. Hypotheses concerning drug metabolism, telomerase up-regulation and telomerase specificity will be tested.

R37AG09383 GREIDER, CAROL W STRUCTURE AND FUNCTION OF TELOMERES I
-PROJECT NUMBER.....5 R37 AG09383-07
INSTITUTEAG FY 97 GREIDER, CAROL W
INITIAL REVIEW GROUP IRGCTY COLD SPRING HARBOR LABORATORY
AWARD AMOUNT..... P O BOX 100, 1 BUNGTOWN RD
COLD SPRING HARBOR, NY 11724
PERFORMING ORGANIZATION: COLD SPRING HARBOR LABORATORY
TITLE STRUCTURE AND FUNCTION OF TELOMERES IN MAMMALIAN AGING
FUTURE YEARS 3
ABSTRACT:

DESCRIPTION: In the last four years, the applicant's research group has documented human telomere shortening, telomerase activation in immortalization and cancer, and established a model that is now widely being tested for the role of telomerase in tumor progression. This group further cloned the RNA component of human and mouse telomerase, and characterized the expression in normal cells and in cancer. Goals in the present application will be: i) to characterize the structure and function of the human telomerase RNA component; ii) to clone the protein components of telomerase; and iii) to determine the pathways that regulate telomerase in normal human cells. When all of the cloned components are in hand, and sufficient information has been gained concerning telomerase regulation, it should become possible to devise direct experimental tests of the role of telomerase in aging and cancer. This basic understanding is essential to evaluate the efficacy and potential toxicity of approaches that are being developed to target telomerase in anti-cancer therapies.

R01AG11728 LUNDBLAD, VICTORIA J TELOMERE REPLICATION AND SENESCENCE I
-PROJECT NUMBER.....2 R01 AG11728-05
INSTITUTEAG FY 97 LUNDBLAD, VICTORIA J
INITIAL REVIEW GROUP IRGCTY BAYLOR COLLEGE OF MEDICINE
AWARD AMOUNT..... ONE BAYLOR PLAZA
HOUSTON, TX 77030
PERFORMING ORGANIZATION: BAYLOR COLLEGE OF MEDICINE
TITLE TELOMERE REPLICATION AND SENESCENCE IN YEAST
FUTURE YEARS 4
ABSTRACT:

DESCRIPTION: The Lundblad lab is using a genetic approach in the budding yeast Saccharomyces cerevisiae to identify potential components or positive regulators of telomerase. During the previous grant period the lab conducted a genetic screen for yeast mutants that displayed a similar phenotype to the previously identified est1- mutant. This mutant displays progressive telomere shortening and a senescence phenotype. The genetic screen resulted in identification of two new EST genes (EST2 and EST3) as well as a novel est-like mutation in a gene previously implicated at the telomere (CDC13). The CDC13 and EST gene products are essential in vivo for telomere replication but are dispensable in vitro for telomerase activity. Thus, they may be essential regulators of telomerase rather than part of the core enzyme. Since Est1p and Cdc13p are both single-strand telomere binding proteins, the PI proposes that these two proteins function to mediate access of telomerase to the chromosomal terminus. Est2p and Est3p could act similarly as components of telomeric chromatin, or as other in vivo regulators of telomerase.

This application proposes to take two inter-related approaches to further analysis of the EST/TLC1 pathway for telomere replication. The first is to extensively characterize each EST gene with the long term goal of determining what each individual protein is doing at the telomere and using this information to ultimately reconstitute telomerase activity in vivo. Specifically, the lab will analyze the biochemical properties of the Est1 protein; look for potential interactions between the individual Est proteins, as well as between the Est proteins and other gene products; determine whether the Est proteins form a complex and are components of either telomeric chromatin or telomerase; reconstitute yeast telomerase activity.

In a parallel approach, the lab will conduct several extensive mutant screens designed to identify additional genes required for telomere function

in yeast including additional EST genes. In this aim the lab will perform a new mutant screen designed to identify additional genes in the EST/TLC pathway for telomere replication; screen the 4900 viable yeast null mutations for changes in telomere length; characterize and clone the genes mutated in a new collection of 25 short and long telomere mutants. The genes identified in these screens will be incorporated into the ongoing genetic and biochemical experiments with the existing EST genes.

U19CA67760 VON HOFF, DANIEL D TELOMERE AND TELOMERASE INTERACTIVE A
-PROJECT NUMBER.....5 U19 CA67760-02
INSTITUTECA FY 96 VON HOFF, DANIEL D
INITIAL REVIEW GROUP IRGSRC UNIV OF TEXAS HLTH SCI CTR
AWARD AMOUNT..... 7703 FLOYD CURL DR
SAN ANTONIO, TX 78284-7862
PERFORMING ORGANIZATION: UNIVERSITY OF TEXAS HLTH SCI CTR SAN ANT
TITLE TELOMERE AND TELOMERASE INTERACTIVE AGENTS
FUTURE YEARS 3
ABSTRACT:

The overall goal of this project is to develop telomere and telomerase interactive agents that improve the survival and quality of life of patients with cancer. Telomeres are repetitive sequences (TTAGGG in humans) at the ends of chromosomes, which have been called "chemical bookends." The integrity of telomeres is vital for cell survival. As cells divide (age), the length of telomeres gradually decreases which leads to chromosome instability. When telomeres become very short, cells undergo a crisis. The cells that survive are immortal and have an increase in the enzyme telomerase. Telomerase is a reverse transcriptase that synthesizes and maintains telomeres. Important to this application are two facts: l) tumor cells have shorter telomeres than do normal cells (because the tumor cells have undergone more divisions); and 2) telomerase is produced in tumor cells (and not in normal cells). These two facts give us unique targets which are different in tumor cells versus normal cells and provide an opportunity to develop agents that affect tumor cells but not normal cells (good selectivity).

We have assembled a group experienced in preclinical and clinical drug development who will tackle these targets using four programs and three cores. Program #1 ("Characterization of Telomerase and Its Inhibitors") will purify telomerase and begin to elucidate the mechanism of action and the structure-activity relationship for inhibitors of telomerase that we have already identified. Program #2 ("Rational Design and Synthesis of Telomerase Inhibitors") will utilize high-field NMR to elucidate unique targets within telomeres and telomerase and use molecular modeling to design drugs that will interact specifically with telomerase. Program #3 ("Telomere and Telomerase Molecular Biology") will both map and clone telomere protein components and isolate and clone telomerase template RNA. These efforts will sharpen our understanding of the enzyme and its template RNA and provide us with even more specific targets. Program #4 ("Telomeres and Telomerase in Primary and Metastatic Human Tumors") will characterize telomere length and telomerase levels in tumors taken directly from patients with breast, lung, head and neck, colon and ovarian cancer. This should identify patient malignancies that can be targeted in future clinical trials with agents developed via this project. In addition, clinically relevant animal model systems will be developed for in vivo testing of our candidate compounds. Finally, there will be three cores including l) an Administrative Core ("A") for coordinating the efforts among our three institutions as well as with the NCI, 2) a

Chemistry Core ("B") for compound synthesis and analytical work, and 3) a Biology Core ("C") for running assays to determine cell viability, in vitro telomerase level. and telomere length. We are confident that this approach to the design and synthesis of telomere/telomerase interactive agents will lead to agents that improve the survival and quality of life of patients with cancer.

R01GM54198 COLLINS, KATHLEEN STRUCTURE AND FUNCTION OF TELOMERASE
-PROJECT NUMBER.....1 R01 GM54198-01
INSTITUTEGM FY 96 COLLINS, KATHLEEN
INITIAL REVIEW GROUP IRGCTY UNIVERSITY OF CALIFORNIA
AWARD AMOUNT.....
BERKELEY, CA 94720-3202
PERFORMING ORGANIZATION: UNIVERSITY OF CALIFORNIA BERKELEY
TITLE STRUCTURE AND FUNCTION OF TELOMERASE
FUTURE YEARS 4
ABSTRACT:

DESCRIPTION: (Adapted from the applicant's abstract): Telomeres protect linear eukaryotic chromosomes from terminal degradation or fusion and can regulate gene expression and possibly cellular senescence. The telomeric DNA simple sequence repeats required for these functions are maintained in a dynamic balance between loss of terminal repeats with genome replication and their addition de novo. The enzyme telomerase, a reverse transcriptase, extends chromosome termini by addition of one strand of simple sequence repeat DNA. The sequence specificity of repeat addition is provided by a template sequence within the integral RNA component of the enzyme. While the template RNA has been identified and isolated from a number of species including ciliates, yeasts, and mammals, none of the protein components of telomerase have been identified from any organism, until now.

The investigator has recently shown that telomerase from the ciliated protozoan Tetrahymena is composed of two protein subunits (p80 and p95) in addition to the 159-nt template RNA. In addition, she has cloned the genes for these two novel proteins. Dr. Collins proposes studies to examine the structure and function of Tetrahymena telomerase in vitro. She plans to determine RNA and protein sequences required for the novel polymerase properties of this enzyme and for RNA- and DNA-protein interactions. Telomerase enzyme mutant in specific properties will then be expressed in Tetrahymena, to test the significance of the affected properties in vivo.

It has been suggested that normal human somatic tissues do not express telomerase activity and that this loss of activity may explain why these cells have only a finite proliferative lifespan. In contrast, malignant cancer cells do express telomerase activity and perhaps due to this can avoid proliferative senescence. Thus while understanding the mechanism of DNA synthesis by this novel ribonucleoprotein polymerase is an important investigation in its own right, these experiments also have direct implications for the improvement of human health.

R29GM50861 ROMERO, DANIEL P STRUCTURAL AND FUNCTIONAL ANALYSIS OF
-PROJECT NUMBER.....5 R29 GM50861-02
INSTITUTEGM FY 96 ROMERO, DANIEL P
INITIAL REVIEW GROUP IRGCTY UNIVERSITY OF MINNESOTA

AWARD AMOUNT..... 435 DELAWARE STREET SE
MINNEAPOLIS, MN 55455
PERFORMING ORGANIZATION: UNIVERSITY OF MINNESOTA TWIN CITIES
TITLE STRUCTURAL AND FUNCTIONAL ANALYSIS OF TELOMERASE RNAS
FUTURE YEARS 3
ABSTRACT:

Telomeres are specialized DNA-protein structures found at the ends of eukaryotic chromosomes which are necessary for the stable maintenance and normal replication of chromosomes. The presence of telomeric sequences at the ends of linear chromosomes is a universal property of all eukaryotic cells. There is a direct correlation between the gradual shortening of telomere length in human somatic cells in vivo and in vitro with the ability of cells to divide. This implies a direct link between telomere length and the aging process. It has been suggested that activation of telomere synthesis may be essential for the growth of immortalized human cells. There are also several lines of evidence linking abnormalities at the telomere with cancer cells.

Telomeric DNA is maintained by a ribonucleoprotein enzyme (telomerase) whose RNA moiety serves as a template, dictating the species-specific telomeric DNA repeat synthesized. Thus, telomerase represents an unusual reverse transcriptase that is template-independent because it contains its own template in the form of an RNA. The experimental system that has been the most amenable to the study of telomeres and telomere synthesis is that of the ciliated protozoans. The aim of the proposed research is to investigate the possible involvement of telomerase RNA (ThR) in the enzymatic activity of telomerase beyond that of a templating function, using the cilate Tetrahymena thermophila as the experimental system. The research plan is logically divided into two objectives. The fist will be to identify the primary and secondary structural features of TER that are invariant throughout evolution, and therefore essential to telomerase activity. This phylogenetic approach will center on ciliate species that are closely allied with T. thermophila, i.e. species that synthesize (GGGGTT) telomeric repeats and are in the suborder Tetrahymenina. Cross-hybridization and PCR will be used to identify and clone additional TER genes. Inclusion of additional TER gene sequences with the fourteen known sequences (seven published) in a phylogenetic analysis will help to more completely define conserved nucleotides that may be of functional importance. This information will help narrow our selection of nucleotides and structural element targeted for mutagenesis to those that are conserved in all tetrahymenine species.

The second objective of the proposed research will be to experimentally test the refined TER secondary structure model. This will be accomplished by site-directed mutagenesis of absolutely conserved primary and secondary structural elements, followed by introduction of the mutated TER gene into Tetrahymena thermophila by DNA-mediated transformation. Mutated TER genes will be cloned into a selectable, Tetrahymena-specific transformation vector and introduced into cells by electroporation. Transformants will be fully characterized for assembly of the plasmid-borne TER transcripts into telomerase and for their ability to synthesize telomeric DNA in vivo. The effect of mutant telomerase RNAs on telomerase activity will also be quantitated in a series of in vitro assays. Telomerase will be partially purified from transformants expressing mutant TER and characterized more completely with respect to number of kinetic parameters (substrate and dNTP binding, Vmax, etc.)

F32AG05781 EPSTEIN, CHARLES B IDENTIFICATION OF COMPONENTS OF YEAST

-PROJECT NUMBER.....1 F32 AG05781-01
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TITLE IDENTIFICATION OF COMPONENTS OF YEAST TELOMERASE
FUTURE YEARS 2
ABSTRACT:

Telomere replication is mediated by a ribonucleoprotein complex called telomerase. While the RNA component of yeast and human telomerase have been cloned the protein components have not. Work initiated by Chuck Epstein in Dr. Zakian's labomtory on physical association between yeast EST1 proteins and telomerase RNA will serve as the initial starting point for experiments in Dr. Shay's laboratory. Dr. Epstein will help to clarify the role of EST1 as well as identify novel potential telomerase components, via their genetic interactions with a novel allele of EST1 recently discovered in the Zakian lab. As soon as is feasible the information obtained from the yeast model system will be applied to determine if proteins similar to EST1 can be identified in human cells. The utility of understanding telomerase structure in a genetic model such as yeast may contribute to our understanding of mammalian telomerase, and help in the development of telomerase targeting drugs. Since telomerase is frequently inactive in somatic cells, but activated in tumor cells, such drugs may have anti-tumor utility.

U19CA677600001 CHEN, SHIH-FONG TELOMERE AND TELOMERASE INTERACTIVE A
-PROJECT NUMBER.....5 U19 CA67760-02 SUB: 0001
INSTITUTECA FY 96 CHEN, SHIH-FONG
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TITLE TELOMERE AND TELOMERASE INTERACTIVE AGENTS
SUB TITLE CHARACTERIZATION OF TELOMERASE AND ITS INHIBITORS
FUTURE YEARS 3
ABSTRACT:

One of the general characteristics of cancer cells is genomic instability. Though it is still unclear what causes this instability, a hypothesis gaining increasing attention is that free chromosome ends, either from chromosome breakage or from loss of the telomere sequences which cap the ends, are prone to illegitimate recombination events. Thus, telomeres provide stability to the chromosomes. However, there appears to be a gradual loss of telomere sequences with each cell division, perhaps because of the end-replication problem. Tumor cells do have shortened telomeres, but they also possess greatly elevated levels of the enzyme telomerase to overcome the end-replication problem, while normal cells do not. Thus, telomerase is an attractive target for new anti-cancer agents because of the expected selectivity for neoplastic cells. Furthermore, we already have preliminary evidence that inhibiting telomerase can kill cancer cells.

We therefore propose to characterize telomerase and its inhibitors in much more detail, in order to select the most effective agents and define their biological effects. Specifically, we propose: I) To isolate, purify, and characterize telomerase(s) from several human tumor types. Telomerase from

selected breast, lung, and colon cancer specimens will be isolated and characterized biochemically, and telomerase from the well-characterized HeLa human tumor cell line will be purified. In addition, we will design new, faster, and more sensitive telomerase activity assays. 2) To elucidate the mechanism and specificity of human tumor telomerase inhibition by selected agents. Agents will include: (a) nucleoside/nucleotide analogs, (b) nonnucleoside reverse transcriptase inhibitors, and (c) antisense molecules. Their ability to inhibit isolated telomerase will be determined. Comparison with other nucleotide processing enzymes such as reverse transcriptase, terminal transferase, and DNA polymerases will help to delineate structural requirements for specific inhibition of telomerase. 3) To compare the formation and repair of drug-induced lesions in telomeres versus bulk DNA. In particular, we will examine the actions of DNA-reactive agents on the structure and function of telomeric DNA. Agents will include: (a) an AT-specific alkylating minor groove binder, (b) a GC-specific DNA intercalating agent, and (c) an enediyne strand scission agent.
From these studies we hope to learn the biochemical and biological consequences of telomerase inhibition, which prototype structures offer the best promise of specific inhibition, and how tumor cells cope with lesions in the telomeres. Overall, this work will shed new light on how interference with the telomere/telomerase system could provide a new and selective therapeutic strategy for human cancer.

R01GM43080 GREIDER, CAROL W CHARACTERIZATION OF THE TETRAHYMENA T
-PROJECT NUMBER.....5 R01 GM43080-07
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FUTURE YEARS 2
ABSTRACT:

Telomerase is a telomere specific DNA polymerase required for the replication of chromosome ends. Conventional DNA polymerases do not complete the replication of the very end of the lagging strand of a linear DNA molecule. Telomerase compensates for this incomplete replication by adding telomere specific repeats onto chromosome ends de novo. Telomerase contains an essential RNA component which specifies the sequence added. Telomerase was first identified in the immortal single cellular protozoan Tetrehymena. Recent experiments suggest that human telomerase regulation may play a role in cancer progression. Telomerase is not active in most human somatic tissue, however in immortalized cells in culture and in cancer tissue telomerase is active. The reactivation of telomerase in tissue culture cells after immortalization suggests that telomerase may be required for the growth of immortalized cells. Thus telomerase has been proposed as a target for anti-cancer therapies. To determine the role of telomerase in cancer, and to help design potential inhibitors it is essential to have a thorough understanding of telomerase biochemistry. Tetrahymena remains the organism of choice from which to study the biochemical mechanism of telomerase. Large quantities of cells can be grown easily and there is 100 to 1000 times more activity than from an equivalent mass of human cells.

The goal of my laboratory is to understand the mechanism and regulation of telomerase. The specific aim of this proposal is to identify and functionally analyze all of the components of the Tetrahymena telomerase.

Telomerase proteins have not been cloned from any organism. We propose to clone and characterize the protein components of the enzyme, dissect the structural requirements for telomerase RNA, use gene replacement to study the requirement for telomerase in vivo and probe the biochemical reaction mechanism of the purified enzyme. The experiments proposed here will in effect work double time; they will establish the mechanism of a new class of DNA polymerase and also may shape the development of new cancer therapies.